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US-PAT-NO: 5871984
DOCUMENT-IDENTIFIER: US 5871984 A

TITLE: Nematode-extracted anticoagulant protein

DATE-ISSUED: February 16, 1999

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US-CL-CURRENT: 514/12; 530/324, 530/350

CLAIMS:

We claim:
1. An isolated protein having anticoagulant activity and having one or more Nematode-extracted Anticoagulant Protein domains ("NAP domains"), wherein each NAP domain includes the sequence:
Cys-A1-Cys-A2-Cys-A3-Cys-A4-Cys-A5-Cys-A6-Cys-A7-Cys-A8-Cys-A9-Cys-A10, wherein
(a) A1 is an amino acid sequence of 7 to 8 amino acid residues;
(b) A2 is an amino acid sequence;
(c) A3 is an amino acid sequence of 3 amino acid residues;
(d) A4 is an amino acid sequence;
(e) A5 is an amino acid sequence of 3 to 4 amino acid residues;
(f) A6 is an amino acid sequence;
(g) A7 is an amino acid residue;
(h) A8 is an amino acid sequence of 11 to 12 amino acid residues;
(i) A9 is an amino acid sequence of 5 to 7 amino acid residues; and
(j) A10 is an amino acid sequence;
wherein each of A2, A4, A6 and A10 has an independently selected number of independently selected amino acid residues and each sequence is selected such that each NAP domain has in total less than about 120 amino acid resiques and wherein said isolated protein is derived from a hematophagous nematode species.
2. The protein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues.
3. The protein of claim 1, wherein A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a is selected from the group consisting of Ala, Arg, Pro, Lys, Ile, His, Leu, and Thr, and A3.sub.b is selected from the group consisting of Lys, Thr, and Arg.
4. The protein of claim 1, wherein A3 is selected from the group consisting of
Glu-Ala-Lys,
Glu-Arg-Lys,
Glu-Pro-Lys,
Glu-Lys-Lys,

Glu-Ile-Thr,
Glu-His-Arg,
Glu-Leu-Lys, and
Glu-Thr-Lys.
5. The protein of claim 1, wherein A4 is an amino acid sequence having a net anionic charge.
6. The protein of claim 1, wherein A7 is Val.
7. The protein of claim 1, wherein A7 is Ile.
8. The protein of claim 1, wherein A8 includes the amino acid sequence A8.sub.a -A8.sub.b -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - [SEQ. ID. NO. 68], wherein
(a) A8.sub.a is the first amino acid residue in A8,
(b) at least one of A8.sub.a and A8.sub.b is selected from the group consisting of Glu or Asp, and
(c) A8.sub.c through A8.sub.g are independently selected amino acid residues.
9. The protein of claim 8, wherein
(a) A8.sub.a is Glu or Asp,
(b) A8.sub.b is an independently selected amino acid residue,
(c) A8.sub.c is Gly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
10. The protein of claim 9, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
11. The protein of claim 8, wherein
(a) A8.sub.a is an independently selected amino acid residue,
(b) A8.sub.b is Glu or Asp,
(c) A8.sub.c is Gly,
(d) A8.sub.d is selected from the group consisting of Phe, Tyr, and Leu,
(e) A8.sub.e is Tyr,
(f) A8.sub.f is Arg, and
(g) A8.sub.g is selected from Asp and Asn.
12. The protein of claim 11, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
13. The protein of claim 8, wherein -A8.sub.c -A8.sub.d -A8.sub.e -A8.sub.f -A8.sub.g - is selected from the group consisting of
Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],
Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],
Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],
Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and
Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73].
14. The protein of claim 1, wherein A10 includes an amino acid sequence selected from the group consisting of
Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74],
Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75],
Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and
Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].
15. The protein of claim 14, wherein A10 includes the amino acid sequence Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74].
16. The protein of claim 15 having a NAP domain with an amino acid sequence of a NAP domain of AcaNAP6 (SEQ. ID. NO. 41).
17. The protein of claim 14, wherein A10 includes the amino acid sequence Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75].
18. The protein of claim 17 having a NAP domain with an amino acid sequence of a NAP domain of AcaNAP49 (SEQ. ID. NO. 42).
19. The protein of claim 14, wherein A10 includes the sequence Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76].
20. The protein of claim 19 having a NAP domain selected from a NAP domain of

AcaNAP23 (SEQ. ID. NO. 43), a NAP domain of AcaNAP24 (SEQ. ID. NO. 44), a NAP domain of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ. ID. NO. 46), a NAP domain of AcaNAP31 (SEQ. ID. NO. 47), and a NAP domain of AceNAP4 (SEQ. ID. NOS. 48 or 49).

21. The protein of claim 14, wherein A11 includes the sequence Met-Glu-Ile-Ile-Thr (SEQ. ID. NO. 77).

22. The protein of claim 21 having a NAP domain with an amino acid sequence selected from a sequence of a NAP domain of AcaNAP48 (SEQ. ID. NOS. 50 or 53), a NAP domain of AcaNAP47 (SEQ. ID. NOS. 51 or 54), a NAP domain of AduNAP7 (SEQ. ID. NOS. 52 or 55), a NAP domain of AduNAP4 (SEQ. ID. NO. 55), a NAP domain of AceNAP5 (SEQ. ID. NO. 57), and a NAP domain of AceNAP7 (SEQ. ID. NO. 58).

23. The protein of claim 1, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.

24. The protein of claim 1, wherein

(a) A3 has the sequence Glu-A3.sub.a -A3.sub.b, wherein A3.sub.a and A3.sub.b are independently selected amino acid residues;

(b) A4 is an amino acid sequence having a net anionic charge;

(c) A7 is selected from the group consisting of Val and Ile;

(d) A8 includes an amino acid sequence selected from the group consisting of

Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 69],

Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 70],

Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 71],

Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 72], and

Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 73]; and

(e) A10 includes an amino sequence selected from the group consisting of

Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74],

Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75],

Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and

Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].

25. The protein of claim 24 having a NAP domain selected from the group consisting of a NAP domain of AcaNAP6 (SEQ. ID. NO. 41), a NAP domain of AcaNAP48 (SEQ. ID. NO. 42), a NAP domain of AcaNAP23 (SEQ. ID. NO. 43), a NAP domain of AcaNAP24 (SEQ. ID. NO. 44), a NAP domain of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ. ID. NO. 46), a NAP domain of AcaNAP31 (SEQ. ID. NO. 47), a NAP domain of AceNAP4 (SEQ. ID. NOS. 48 or 49), a NAP domain of AcaNAP45 (SEQ. ID. NOS. 50 or 53), a NAP domain of AcaNAP47 (SEQ. ID. NOS. 51 or 54), a NAP domain of AduNAP7 (SEQ. ID. NOS. 52 or 55), a NAP domain of AduNAP4 (SEQ. ID. NO. 55), a NAP domain of AceNAP5 (SEQ. ID. NO. 57), and a NAP domain of AceNAP7 (SEQ. ID. NO. 58).

26. The protein of claim 25, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligomosomoides polygyrus.

27. The protein of claim 1, wherein

(a) A3 is selected from the group consisting of

Glu-Ala-Lys,

Glu-Arg-Lys,

Glu-Pro-Lys,

Glu-Lys-Lys,

Glu-Ile-Thr,

Glu-His-Arg,

Glu-Leu-Lys, and

Glu-Thr-Lys;

(b) A4 is an amino acid sequence having a net anionic charge;

(c) A7 is Val or Ile;

(d) A8 includes an amino acid sequence selected from the group consisting of

A8.sub.a -A8.sub.b -Gly-Phe-Tyr-Arg-Asp [SEQ. ID. NO. 78],

A8.sub.a -A8.sub.b -Gly-Phe-Tyr-Arg-Asn [SEQ. ID. NO. 79],

A8.sub.a -A8.sub.b -Gly-Tyr-Tyr-Arg-Asp [SEQ. ID. NO. 80],

A8.sub.a -A8.sub.b -Gly-Tyr-Tyr-Arg-Asn [SEQ. ID. NO. 81], and

A8.sub.a -A8.sub.b -Gly-Leu-Tyr-Arg-Asp [SEQ. ID. NO. 82], wherein at least one of A8.sub.a and A8.sub.b is Glu or Asp;

(e) A9 is an amino acid sequence of five amino acid residues; and

(f) A10 includes an amino acid sequence selected from the group consisting of

Glu-Ile-Ile-His-Val [SEQ. ID. NO. 74],

Asp-Ile-Ile-Met-Val [SEQ. ID. NO. 75],

Phe-Ile-Thr-Phe-Ala-Pro [SEQ. ID. NO. 76], and

Met-Glu-Ile-Ile-Thr [SEQ. ID. NO. 77].

28. The protein of claim 27 having a NAP domain selected from the group consisting of a NAP domain of AcaNAP6 (SEQ. ID. NO. 41), a NAP domain of AcaNAP48 (SEQ. ID. NO.

42), a NAP domain of AcaNAP23 (SEQ. ID. NO. 43), a NAP domain of AcaNAP24 (SEQ. ID. NO. 44), a NAP domain of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ. ID. NO. 46), a NAP domain of AcaNAP31 (SEQ. ID. NO. 47), a NAP domain of AceNAP4 (SEQ. ID. NOS. 48 or 49), a NAP domain of AcaNAP45 (SEQ. ID. NOS. 50 or 53), a NAP domain of AcaNAP47 (SEQ. ID. NOS. 51 or 54), a NAP domain of AduNAP7 (SEQ. ID. NOS. 52 or 56), a NAP domain of AduNAP4 (SEQ. ID. NO. 55), a NAP domain of AceNAP5 (SEQ. ID. NO. 57), and a NAP domain of AceNAP7 (SEQ. ID. NO. 58).

29. The protein of claim 27, wherein said nematode species is selected from the group consisting of Ancylostoma caninum, Ancylostoma ceylanicum, Ancylostoma duodenale, Necator americanus, and Heligmosomoides polygyrus.

30. A pharmaceutical composition comprising a protein of claim 1.

31. A pharmaceutical composition comprising a protein of claim 24.

32. A pharmaceutical composition comprising a protein of claim 27.

33. A method of inhibiting blood coagulation comprising administering a protein of claim 1 with a pharmaceutically acceptable carrier.

34. A method of inhibiting blood coagulation comprising administering a protein of claim 24 with a pharmaceutically acceptable carrier.

35. A method of inhibiting blood coagulation comprising administering a protein of claim 27 with a pharmaceutically acceptable carrier.

36. A protein of claim 1, wherein said protein has two NAP domains.

37. A protein of claim 24, wherein said protein has two NAP domains.

38. A protein of claim 27, wherein said protein has two NAP domains.

39. An isolated protein having anticoagulant activity selected from the group consisting of AcaNAP6 (SEQ. ID. NO. 41), AcaNAP48 (SEQ. ID. NO. 42), AcaNAP23 (SEQ. ID. NO. 43), AcaNAP24 (SEQ. ID. NO. 44), AcaNAP25 (SEQ. ID. NO. 45), AcaNAP44 (SEQ. ID. NO. 46), AcaNAP31 (SEQ. ID. NO. 47), AceNAP4 (SEQ. ID. NO. 62), AcaNAP45 (SEQ. ID. NO. 63), AcaNAP47 (SEQ. ID. NO. 64), AduNAP7 (SEQ. ID. NO. 65), AduNAP4 (SEQ. ID. NO. 55), AceNAP5 (SEQ. ID. NO. 57), and AceNAP7 (SEQ. ID. NO. 58).

40. A pharmaceutical composition comprising a protein having a NAP domain selected from the group consisting of a NAP domain of AcaNAP6 (SEQ. ID. NO. 41), a NAP domain of AcaNAP48 (SEQ. ID. NO. 42), a NAP domain of AcaNAP23 (SEQ. ID. NO. 43), a NAP domain of AcaNAP24 (SEQ. ID. NO. 44), a NAP domain of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ. ID. NO. 46), a NAP domain of AcaNAP31 (SEQ. ID. NO. 47), a NAP domain of AceNAP4 (SEQ. ID. NOS. 48 or 49), a NAP domain of AcaNAP45 (SEQ. ID. NOS. 50 or 53), a NAP domain of AcaNAP47 (SEQ. ID. NOS. 51 or 54), a NAP domain of AduNAP7 (SEQ. ID. NOS. 52 or 56), a NAP domain of AduNAP4 (SEQ. ID. NO. 55), a NAP domain of AceNAP5 (SEQ. ID. NO. 57), and a NAP domain of AceNAP7 (SEQ. ID. NO. 58).

41. A method of inhibiting blood coagulation comprising administering a protein having a NAP domain selected from the group consisting of a NAP domain of AcaNAP6 (SEQ. ID. NO. 41), a NAP domain of AcaNAP48 (SEQ. ID. NO. 42), a NAP domain of AcaNAP23 (SEQ. ID. NO. 43), a NAP domain of AcaNAP24 (SEQ. ID. NO. 44), a NAP domain of AcaNAP25 (SEQ. ID. NO. 45), a NAP domain of AcaNAP44 (SEQ. ID. NO. 46), a NAP domain of AcaNAP31 (SEQ. ID. NO. 47), a NAP domain of AceNAP4 (SEQ. ID. NOS. 48 and 49), a NAP domain of AcaNAP45 (SEQ. ID. NOS. 50 and 53), a NAP domain of AcaNAP47 (SEQ. ID. NOS. 51 and 54), a NAP domain of AduNAP7 (SEQ. ID. NOS. 52 and 56), a NAP domain of AduNAP4 (SEQ. ID. NO. 55), a NAP domain of AceNAP5 (SEQ. ID. NO. 57), and a NAP domain of AceNAP7 (SEQ. ID. NO. 58).

42. A protein having two NAP domains, wherein said protein is selected from the group consisting of AceNAP4 [SEQ. ID. NO. 62], AcaNAP45 [SEQ. ID. NO. 63], AcaNAP47 [SEQ. ID. NO. 64], and AduNAP7 [SEQ. ID. NO. 65].